

What is claimed:

1. An isolated nucleic acid which comprises a nucleotide segment having a sequence encoding a complex comprising a viral surface protein and a corresponding viral transmembrane protein wherein the complex contains one or more mutations in amino acid sequence that enhance the stability of the complex formed between the viral surface protein and the viral transmembrane protein.
2. The isolated nucleic acid of claim 1, wherein the virus is a lentivirus.
3. The isolated nucleic acid of claim 1, wherein the virus is the human immunodeficiency virus.
4. The isolated nucleic acid of claim 3, wherein the human immunodeficiency virus is a primary isolate.
5. The isolated nucleic acid of claim 3, wherein the human immunodeficiency virus is HIV-1_{JR-FL}, HIV-1_{DH123}, HIV-1_{Gun-1}, HIV-1_{89.6}, or HIV-1_{HXB2}.
6. The isolated nucleic acid of claim 3, wherein the viral surface protein is gp120 or a modified form of gp120, wherein the modification alters the immunogenicity of the molecule relative to wild type gp120.
7. The isolated nucleic acid of claim 6, wherein the modified gp120 molecule is characterized by the absence of one or more variable loops present in wild type gp120.

8. The isolated nucleic acid of claim 7, wherein the variable loop comprises V1, V2, or V3.
9. The isolated nucleic acid of any one of claims 6-8, wherein the modified gp120 molecule is characterized by the absence or presence of one or more canonical glycosylation sites present absent or absent in wild type gp120.
10. The isolated nucleic acid of claim 9, wherein one or more canonical glycosylation sites are absent from the V1V2 region of the gp120 molecule.
11. The isolated nucleic acid of any one of claims 3-10, wherein the transmembrane protein is gp41 or a modified form of gp41, wherein the modification alters the immunogenicity of the molecule relative to wildtype gp41.
12. The isolated nucleic acid of claim 11, wherein the transmembrane protein is the gp41 ectodomain.
13. The isolated nucleic acid of claim 11 or 12, wherein the transmembrane protein is modified by the absence or presence of one or more canonical glycosylation sites absent or present in the wild type gp120.
14. The isolated nucleic acid of any one of claims 1-13, wherein the stabilization of the complex is achieved by one or more cysteine-cysteine bonds that are formed between the surface and transmembrane proteins and that are not present in the corresponding wildtype complex.

15. The isolated nucleic acid of claim 14, wherein one or more amino acids which are adjacent to or which contain an atom within 5 angstroms of an introduced cysteine are mutated to a noncysteine residue.
16. The isolated nucleic acid of claim 14 or 15, wherein one or more cysteines in gp120 or modified form of gp120 are disulfide linked to one or more cysteines in gp41 or modified form of gp41.
17. The isolated nucleic acid of claim 16, wherein a cysteine in the C5 region of gp120 or modified form of gp120 is disulfide linked to a cysteine in the ectodomain of gp41.
18. The isolated nucleic acid of claim 16, wherein the disulfide bond is formed between a cysteine introduced by an A492C mutation in gp120 and a T596C mutation in gp41.
19. The isolated nucleic acid molecule of claim 1 which is cDNA.
20. The isolated nucleic acid molecule of claim 1 which is genomic DNA.
21. The isolated nucleic acid molecule of claim 1 which is RNA.
22. A replicable vector comprising the nucleic acid of claim 1.
23. A plasmid, cosmid, λ phage or YAC containing the nucleic acid of claim 1.

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24. The plasmid of claim 23 designated PPI4.
25. A host cell containing the vector of claim 22.
26. The cell of claim 25 which is a eukaryotic cell.
27. The cell of claim 25 which is a bacterial cell.

5 28. A vaccine which comprises the isolated nucleic acid of claim 1.

29. A vaccine which comprises a therapeutically effective amount of the nucleic acid of claim 1.

sub
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a1

30. A vaccine which comprises a therapeutically effective amount of the protein encoded by the nucleic acid of claim 1.

15 31. A method of treating a viral disease which comprises immunizing a virally infected subject with the vaccine of claim 29 or 30 or a combination thereof, thereby treating the subject.

32. A vaccine which comprises a prophylactically effective amount of the nucleic acid of claim 1.

sub
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a2

33. A vaccine which comprises a prophylactically effective amount of the protein encoded by the nucleic acid of claim 1.

25 34. A method of reducing the likelihood of a subject becoming infected with a virus comprising administering the vaccine of claim 32 or 33 or a combination thereof, thereby reducing the likelihood of the subject becoming infected with the virus.

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35. A vaccine comprising the nucleic acid of any one of claims 3-18.
36. A vaccine which comprises a therapeutically effective amount of the nucleic acid of any one of claims 3-18.
37. A vaccine which comprises a therapeutically effective amount of the protein encoded by the nucleic acid of any one of claims 3-18.
38. A method of treating an HIV-1 infected subject which comprises immunizing the subject with the vaccine of claim 36 or 37 or a combination thereof, thereby treating the subject.
39. A vaccine which comprises a prophylactically effective amount of the nucleic acid of any one of claims 3-18.
40. A vaccine which comprises a prophylactically effective amount of the protein encoded by the nucleic acid of any one of claims 3-18.
41. A method of reducing the likelihood of a subject becoming infected with HIV-1 comprising administering the vaccine of claim 39 or 40 or a combination thereof, thereby reducing the likelihood of the subject becoming infected with HIV-1.
42. The vaccine of claim 35, wherein the vaccine comprises a recombinant subunit protein, a DNA plasmid, a replicating viral vector, a non-replicating viral vector, or a combination thereof.

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43. A method of reducing the severity of HIV-1 disease in a subject comprising administering the vaccine of claim 39 or 40 or a combination thereof, prior to exposure of the subject to HIV-1, thereby reducing the severity of HIV-1 disease or AIDS in the subject upon subsequent exposure to HIV-1.

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44.

A viral envelope protein comprising a viral surface protein and a corresponding viral transmembrane protein wherein the viral envelope protein contains one or more mutations in amino acid sequence that enhance the stability of the complex formed between the viral surface protein and transmembrane protein.

HIV-1 ONLY!

W.D.

F.W.A.B.

102(6): PARZAN

What type of (?)

15 45.

other agent:
multimer?

102(6): PARZAN

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Sub
A3

46.

A mutant HIV-1 envelope protein which is encoded by the nucleic acid of any one of claims 3-18.

47.

The protein of claim 44 or complex of claim 45 which is linked to at least one other protein or protein fragment to form a fusion protein.

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A purified protein of any one of claims 44-46.

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A vaccine which comprises a therapeutically effective amount of the protein of claim 44 or the complex of claim 45.

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A vaccine which comprises a prophylactically

effective amount of the protein of claim 44 or the complex of claim 45.

51. A method of stimulating or enhancing in a subject production of antibodies which recognize the protein of claim 44 or complex of claim 45.
52. An antibody, antibody chain, fragment or derivative thereof isolated or identified using the viral envelope protein encoded by the recombinant nucleic acid of claim 1.
53. The antibody of claim 52, wherein the antibody is of the IgM, IgA, IgE or IgG class or subclasses thereof.
54. The antibody fragment of claim 52 which includes but is not limited to Fab, Fab' (Fab')₂, Fv and single chain antibodies.
55. The isolated antibody light chain of the antibody of claim 52, or fragment or oligomer thereof.
56. The isolated antibody heavy chain of the antibody of claim 52, or fragment or oligomer thereof.
57. One or more complementarity determining regions of the antibody of claim 52.
58. The antibody of claim 52 which is derivatized such as by the addition of a fluorescent moiety, a radionuclide, an enzyme, a toxin, or an affinity ligand such as biotin.
59. The antibody of claim 52 wherein the antibody is a human antibody.

60. The antibody of claim 52 or 59, wherein the antibody is a monoclonal antibody.
61. The antibody of claim 52, wherein the antibody is a humanized antibody.
- 5 62. The antibody of claim 52 or any one of claims 59-61, wherein the viral envelope protein is derived from HIV-1.
63. An isolated nucleic acid molecule encoding the antibody of claim 52 or any one of claims 59-61, wherein the nucleic acid molecule is RNA, genomic DNA or cDNA.
- 10 64. The isolated nucleic acid of claim 63, wherein the viral envelope protein is derived from HIV-1.
65. An agent capable of inhibiting the binding of the antibody of claim 52.
- 15 66. A method of reducing the likelihood of an HIV-1-exposed subject from becoming infected with HIV-1 comprising administering the antibody of claim 62 or the isolated nucleic acid of claim 64, thereby reducing the likelihood of the HIV-1 exposed subject from becoming infected with HIV-1.
- 20 67. A method of treating a subject infected with HIV-1 comprising administering the antibody of claim 62 or the isolated nucleic acid of claim 64, thereby treating the subject.
- 25 68. An agent capable of binding the mutant viral envelope protein encoded by the recombinant nucleic acid molecule of claim 1.

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69. The agent of claim 68 which inhibits viral infection.
70. The agent of claim 69, wherein the viral envelope protein is derived from HIV-1.
- 5 71. A method for determining whether a compound is capable of inhibiting a viral infection comprising:
- 10 (A) contacting an appropriate concentration of the compound with the mutant viral envelope protein encoded by the nucleic acid of claim 1 under conditions permitting binding of the compound to said protein;
- 15 (B) contacting the resulting complex with a reporter molecule under conditions that permit binding of the reporter molecule to the mutant viral envelope protein in the absence of the compound;
- (C) measuring the amount of bound reporter molecule; and
- 20 (D) comparing the amount of bound reporter molecule in step (c) with the amount determined in the absence of the compound, a decrease in the amount indicating that the compound is capable of inhibiting infection by the virus.
- 25 72. The method of claim 71, wherein the reporter molecule is an antibody or derivative thereof.
73. The method of claim 71, wherein the reporter molecule comprises one or more host cell viral receptors or molecular mimics thereof.

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74. A method for determining whether a compound is capable of inhibiting a viral infection which comprises:

- 5 (a) contacting an appropriate concentration of the compound with a host cell viral receptor or molecular mimic thereof under conditions that permit binding of the compound and receptor or receptor mimic in the absence of the compound;
- 10 (b) contacting the resulting complex with the mutant viral envelope protein encoded by the recombinant nucleic acid of claim 1 under conditions that permit binding of the envelope protein and receptor or receptor mimic in the absence of the compound;
- 15 (c) measuring the amount of binding of envelope protein to receptor or receptor mimic;
- 20 (d) comparing the amount of binding determined in step (c) with the amount determined in the absence of the compound, a decrease in the amount indicating that the compound is capable of inhibiting infection by the virus.

75. The method of any one of claims 71-74 wherein the virus is HIV-1.

25 76. The method of claim 71 or 72, wherein the host cell viral receptor is CD4, CCR5, CXCR4 or combinations or molecular mimics thereof.

77. The method of any one of claims 71-76, wherein the compound was not previously known.

78. A compound determined to be capable of inhibiting

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a viral infection by the method of any one of claims 71-76.

79. A pharmaceutical composition comprising an amount of the compound effective to inhibit viral infection determined by the method of any one of claims 71-76 to be capable of inhibiting viral infection and a pharmaceutically acceptable carrier.

80. The pharmaceutical composition of claim 79, wherein the viral infection is HIV-1 infection.

81. A viral envelope protein comprising a viral surface protein and a corresponding viral transmembrane protein wherein the viral envelope protein contains one or more mutations in amino acid sequence that enhance the stability of the complex formed between the viral surface protein and transmembrane protein, wherein the surface protein and transmembrane protein are encoded by different nucleic acids.

82. A complex comprising a viral surface protein and a corresponding viral transmembrane protein of a viral envelope protein wherein the viral envelope protein contains one or more mutations in amino acid sequence that enhance the stability of the complex formed between the viral surface protein and transmembrane protein, wherein the surface protein and transmembrane protein are encoded by different nucleic acids.

83. An antibody which binds to the protein of claim 44 or the complex of claim 45 but does not cross react with the individual monomeric surface protein or the individual monomeric transmembrane

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103: PARZAN

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103 PARZAN

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protein.

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84. The antibody of claim 83 capable of binding to the HIV-1 virus.
85. A virus-like particle which comprises the complex of claim 45.
86. The virus-like particle of claim 85, further comprising an immunodeficiency virus gag protein.

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